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ORIGINAL ARTICLE

Tensiomyography parameters and serum biomarkers after eccentric exercise of the elbow flexors

Jan-Frieder Harmsen[1] · Alexander Franz[1] · Constantin Mayer[2] · Christoph Zilkens[1] · Bettina Alexandra Buhren[1] · Holger Schrumpf[1] · Rüdiger Krauspe[1] · Michael Behringer[3]

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Abstract

Purpose The tensiomyography (TMG) technique is increasingly used to determine muscle contractile properties in exercise and injury management. The present study investigated the informative value of TMG parameters in correlation with commonly used (creatine kinase, CK; myoglobin, Mb) and novel candidate biomarkers of muscle damage (heart-type fatty acid-binding protein, h-FABP; high-mobility group box 1, HMGB1).

Methods Ten untrained men performed 6 × 10 eccentric contractions of the elbow flexors at 110% of the concentric one repetition maximum. CK, Mb, h-FABP, HMGB1, arm circumference, pain and TMG data, including maximal displacement (D_m) and temporal outcomes as the contraction time (T_c), sustained time (T_s), delay time (T_d), and relaxation time (T_r), were assessed pre-exercise, post-exercise, 20 min, 2 h and on the consecutive 3 days post-exercise.

Results CK and h-FABP significantly increased beginning at 24 h, Mb already increased at 2 h ($p < 0.05$). HMGB1 was only increased immediately post-exercise ($p = 0.02$). T_c and T_d remained unchanged, whereas T_s and T_r were significantly slower beginning at 24 h ($p < 0.05$). D_m was decreased within the first 24 h and after 72 h ($p < 0.01$). The % change from pre-exercise correlated for D_m with CK, Mb, and h-FABP the highest at 48 h ($r = -0.95$, -0.87 and -0.79; $p < 0.01$) and for h-FABP with CK and Mb the highest at 24 h ($r = 0.96$ and 0.94, for all $p < 0.001$).

Conclusion This study supports the correlation of TMG parameters with muscle damage markers after eccentric exercise. Therefore, TMG could represent a non-invasive and cost effective alternative to quantify the degree of muscle damage after exercise interventions.

Keywords EIMD · Creatine kinase · Myoglobin · h-FABP · HMGB1 · Nonresponder

Abbreviations

ANOVA	Analysis of variance
ATP	Adenosine triphosphate
CK	Creatine kinase
D_m	Maximal displacement
EIMD	Exercise-induced muscle damage
h-FABP	Heart-type fatty acid binding protein
HMGB1	High-mobility group box 1
Mb	Myoglobin
MVC	Maximal voluntary contraction
NR	Nonresponders
POCT	Point-of-care-testing
TMG	Tensiomyography
T_c	Contraction time
T_d	Delay time
T_r	Relaxation time
T_s	Sustained time
VAS	Visual analog scale
1-RM	One repetition maximum

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✉ Jan-Frieder Harmsen
janfrieder.harmsen@gmail.com

[1] Department of Orthopedics, University Hospital Duesseldorf, Moorenstrasse 5, 40225 Duesseldorf, Germany

[2] Department of Orthopedics, University Hospital Essen, Essen, Germany

[3] Faculty of Sport Sciences, Goethe University Frankfurt, Frankfurt, Germany

Introduction

Eccentric contractions are widely used in muscle research to intentionally provoke exercise-induced muscle damage (EIMD) (Clarkson and Ebbeling 1988; Hunter et al. 2012; Kim and Lee 2015). Eccentric contractions will alter muscle homeostasis to a greater extent than concentric and isometric contractions as higher loads work on the muscle. Thus, cross-bridge cycling of myosin and actin is forced to detach leading to streaming alterations of the *Z* lines (Fernandez-Gonzalo et al. 2012). As the resulting muscle damage is normally accompanied by an increase in membrane permeability (Clarkson and Ebbeling 1988), muscular proteins such as creatine kinase (CK), myoglobin (Mb), and others are able to drain into blood circulation (Hyldahl and Hubal 2014), and are, therefore, frequently used as indirect markers of EIMD.

In the last two centuries, especially, serum CK became very popular to monitor EIMD over time due to its simple identification and low cost of assays (Koch et al. 2014). However, there are major concerns about using serum CK as the only indirect marker in terms of muscle damage because of its great inter-individual variability, i.e., due to factors such as training level (Newton et al. 2008) or body mass index (Kim and Lee 2015). Moreover, Nosaka et al. (2006) found that serum CK activity was poorly correlated with parameters of muscle function after eccentric exercise of the elbow flexors. This multifactorial variability of CK and other muscle damage biomarkers has led practitioners as well as researchers to rely more on the assessment of functional outcomes when quantifying the extent of muscle damage.

Therefore, in addition to strength measurements, the assessment of muscle contractile capacities by tensiomyography (TMG) has been proposed to be useful to measure muscle function quantitatively after exercise [for recent review, see Macgregor et al. (2018)]. First introduced as an involuntary measure of muscle contractility, i.e., muscle contraction time (T_c) and maximal displacement (D_m), TMG has been proven to be an effective tool for detecting EIMD (Hunter et al. 2012; Franz et al. 2017). Especially, a decrease in the D_m parameter was shown to be correlated with a reduction of maximal voluntary contraction (MVC) strength and a concurrent increase of CK after eccentric contractions of the elbow flexors for up to 4 days (Hunter et al. 2012; Franz et al. 2017). However, the correlation of TMG parameters to other biomarkers of EIMD remains to be tested.

Thus, in this study, we tested the correlation of TMG data with the well-established markers CK and Mb as well as with heart-type fatty acid-binding protein (h-FABP) and high-mobility group box 1 (HMGB1). The latter two have both been successfully used in the clinical setting for the purpose of quantifying myocardial injury [for recent review, see Ye et al. (2018)] and trauma-induced soft-tissue damage (Peltz et al. 2009), respectively. Therefore, another objective of this study was to evaluate the practical usefulness of these two alternative biomarkers in EIMD monitoring. For this purpose, they were additionally correlated with CK, Mb, pain perception, and muscle swelling after eccentric exercise of the elbow flexors.

Materials and methods

Participants

Ten male participants (age 23 ± 3 years; height 184 ± 9 cm; weight 77 ± 10 kg) volunteered for this study. None of the participants was involved in regular strength training during the 6 months before the start of the study. Participants were free of acute and chronic diseases and musculoskeletal injury. To avoid any potential influence on the investigated parameters, any medication or intake of dietary supplements were defined as exclusion criteria. Before the investigation, written informed consent was obtained from all participants included. The study was approved by the local Research Ethics Committee and was performed according to the Declaration of Helsinki.

Study design

All participants reported to the laboratory for two testing sessions and follow-up measurements, 2 weeks apart. During the first visit, participants' individual concentric one repetition maximum (1-RM) of the elbow flexors was determined. During the second visit, the participants performed an eccentric exercise protocol targeting the elbow flexors. For comprehensive monitoring of EIMD, muscle swelling, muscle contractility, subjective pain intensity, and serum concentrations of four different muscle damage markers (CK, Mb, h-FABP, and HMGB-1) were measured at the following timepoints: pre-exercise (pre-Ex.), immediately post-exercise (post-Ex.), 20 min post-exercise (post-20 min), 2 h post-exercise (post-2 h), 24 h post-exercise (post-24 h), 48 h post-exercise (post-48 h), and 72 h post-exercise (post-72 h). All participants were requested to refrain from strenuous physical activity 48 h prior to testing as well as throughout the testing days (Fig. 1).

1-RM test

Two weeks before the eccentric exercise protocol, the concentric 1-RM of the elbow flexors was determined using bilateral biceps curls. Therefore, participants were standing

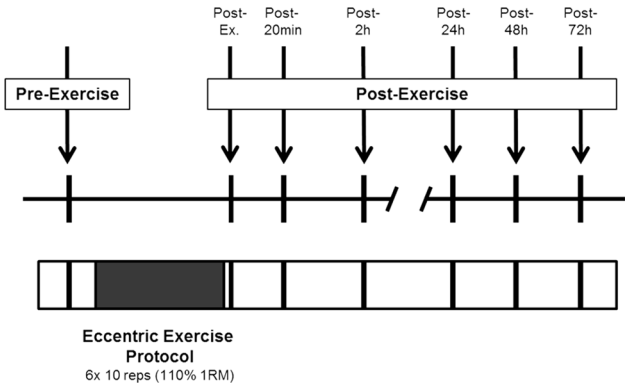

Fig. 1 Study design. All outcomes were measured on seven occasions indicated by bold arrows

in an upright position, with the back leaning against a wall. Throughout the execution of the barbell lift, both elbows continuously held contact with the wall. The 1-RM protocol used in a study of Thiebaud et al. (2013) for unilateral dumbbell curls was modified for bilateral biceps curls with a 10 kg barbell: the warm-up consisted of five-to-six repetitions with a low load (approximately 30–40% of predicted 1-RM). After warming up, the load was set at ~ 80% of the predicted 1-RM. Following each successful lift (the investigator took the barbell at the top of the movement, so that only the concentric action was performed by the participant), the load was increased by ~ 5% with an accuracy of 1 kg until the subject failed to lift the load through the whole range of motion. A test was considered valid if the subject used proper form and completed the whole lift in a controlled manner without assistance. On average, five trials were required to complete a 1-RM test (5 min rest between each attempt).

Eccentric exercise protocol

Modified from Foley et al. (1999), the eccentric exercise protocol consisted of bilateral biceps curls using a barbell (ScSports, Emmerich, Germany) and was performed with 6 × 10 repetitions at 110% of participants individual concentric 1-RM. A bilateral approach was chosen to use one arm for TMG measurements and the other one for blood samplings of the antecubital vein. In addition, unilateral eccentric exercise was found to reduce neuromuscular activity and physical work capacity of the non-exercised homologous muscle in the contralateral limb (Hedayatpour et al. 2018) impeding its use as a control condition. The rest between sets was 1 min. The basic positioning of the participants was the same as used in the 1-RM test. To load the muscles eccentrically only, following each repetition two external assistants lifted up the barbell to a full elbow flexion (~ 50°). Thus, the full range of motion was used for each eccentric contraction. The duration of each eccentric repetition was set to 2 s controlled by a metronome, subsequently followed by a rest of 2 s during the passive uplift of the bar ("concentric phase").

Blood sampling and analysis

For evaluation of eccentric EIMD, venous blood samples from the antecubital vein were collected at each corresponding timepoint (Fig. 1). Serum CK activity (Roche Diagnostic, Mannheim, Germany), as well as concentrations of Mb (Abcam, Cambridge, MA) and HMGB1 (IBL International GmbH, Hamburg, Germany), was measured by enzymatic kinetic assay methods using a Hitachi 912 Automatic Analyzer (Roche Diagnostic). For detection of h-FABP, the point-of-care-testing (POCT) system named concile®Ω100 (concile GmbH, Freiburg, Germany) was used.

Muscle swelling and pain

The development of muscle swelling was monitored by assessing the circumference of the dominant arm at the mid-portion of the upper arm, defined as 50% of the length between the acromion process and the lateral epicondyle of the humerus. Induced by palpation, perceived pain at each of the defined timepoints was assessed using a continuous visual analog scale (VAS) consisting of a 100 mm horizontal line labeled with the words "no pain" (0 mm) and "maximally tolerable pain" (100 mm).

Muscle contractility

The contractile properties of the biceps brachii muscle of the dominant arm were assessed by tensiomyography (TMG) (TMG-BMC, Ljubljana, Slovenia). Participants were seated on a chair, while the investigated arm was taken into a prefabricated thermoplastic cast. Thus, the investigated arm was reliably fixed in a neutral position (i.e., 90-degree elbow flexion). The arm was additionally laid on an adaptable arm support to ensure that the shoulder joint was kept in a neutral position during the measurement.

According to the TMG manual, two self-adhesive bipolar electrodes (Compex Medical SA, Ecublens, Switzerland) were placed on the investigated muscle. The proximal electrode was placed underneath the region, where the pectoralis major- and the deltoid muscle overlap the biceps muscle (Crista tuberculi minoris), while the distal electrode was placed close to the distal tendon of the muscle. The attachment for the displacement-measuring sensor (GK40, Panoptik, Ljubljana, Slovenia) was anatomically determined as a point of maximal muscle belly contraction, detected by manual palpation during pre-measurements of electrically evoked muscle contractions. Both the position of the electrodes and the measuring point were marked with permanent

ink to assure that the same location was used in consecutive measurements. Throughout the measurement, the sensor was placed on the skin perpendicularly to the muscle surface. The participants were instructed to remain relaxed to ensure minimum tension in the investigated arm. A single monophasic square wave with 1 ms pulses was delivered from a TMG-S1 stimulator (EMF-Furlan and Co. do.o., Ljubljana, Slovenia). To receive maximal peripheral mechanical muscle responses (TMG manual), the stimulation was increased by 10 mA at a frequency of 10 s intervals to minimize effects of fatigue and potentiation. The stimulation was gradually increased until no further displacement of the muscle belly was observed.

In the present study, we analyzed five parameters, which were calculated based on the radial displacement curve over time: The maximal radial displacement (D_m) is expressed in millimeters (mm) and measures muscle stiffness and contractile force (Simunič et al. 2011; García-Manso et al. 2012). The duration between 10 and 90% of D_m reflects the contraction time (T_c) and is associated with the muscle fiber type composition and the speed of force generation (Simunič et al. 2011). The delay time (T_d) represents the time in milliseconds (ms) between the electrical impulse and 10% of the maximal displacement. The sustained time (T_s) is the calculated time, where the evoked contraction is maintained, defined by the time of a muscle response greater than 50% of D_m. Finally, the relaxation time (T_r) mirrors the period of time for D_m to decrease from 90 to 50% (Fig. 2). The latter three have not yet been found to correlate with functional outcomes or muscle damage markers, respectively. The reliability of TMG for the biceps brachii muscle has been shown (Krizaj et al. 2008).

Statistics

Because of the small sample size ($n = 10$), normal distribution was rejected. Consequently, the Friedman test as a non-parametric alternative to the one-way ANOVA with repeated measures was used and Dunn's multiple comparisons tests were further conducted to detect significant changes over time. The Spearman rank correlation was used to establish the relationship between the changes of D_m as well as all temporal outcomes (T_r, T_s, T_d, and T_c) vs. CK, Mb, h-FABP, HMGB1, arm circumference, and pain from Pre-Ex. to all post-measurements. h-FABP and HMGB1 were also correlated with the other muscle damage markers. The applied categorization of r values was defined by Hopkins et al. (2009). Statistical significance was set to $\alpha < 0.05$ for all analyses and means with respective standard deviations are used to present data in figures and the running text. Vertical bars in figures represent the standard deviations. All statistical analyses were performed using the GraphPad Prism 6 software package (GraphPad Software, San Diego, CA, USA).

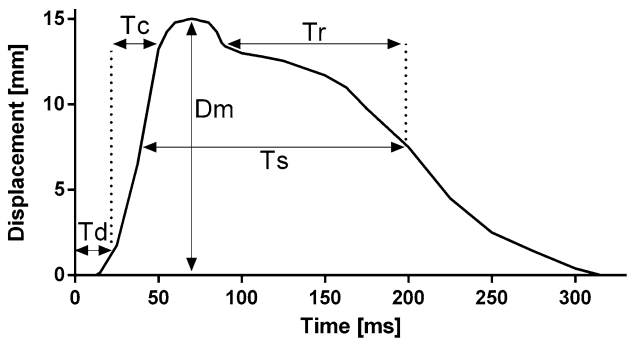

Fig. 2 Tensiomyography parameters definition. T_d delay time, T_c contraction time, D_m maximal displacement, T_s sustained time, T_r relaxation time

Results

1-RM

The mean 1-RM was 42.4 ± 6.8 kg and 46.6 ± 7.5 kg was used as 110% of the concentric 1-RM for the mechanical load during the eccentric exercise protocol.

Serum creatine kinase and myoglobin

At baseline, CK values were 168.5 ± 137.9 U L^{-1}. After eccentric exercise, CK was significantly increased at 24 h (mean change of 6978%, $p = 0.003$), at 48 h and 72 h (20,632% and 30,700%, $p < 0.001$) compared to baseline. The individual maximum value was 88,505 U L^{-1}. The baseline concentration of serum Mb was 1.9 ± 1.4 ng mL^{-1}. Mb was significantly elevated at 2 h (484%, $p = 0.006$), 24 h (4005%), 48 h, and 72 h (9111% and 7237%, $p < 0.001$) (Table 1).

Alternative serum markers for EIMD (h-FABP, HMGB1)

The baseline concentration of serum h-FABP was 2.8 ± 0.6 ng mL^{-1}. Serum h-FABP was significantly elevated at 24 h (718%, $p = 0.019$), 48 h, and 72 h (1975% and 1375%, $p < 0.001$). Serum HMGB1 increased significantly immediately after exercise (25 ± 10.3 ng mL^{-1}, $p = 0.016$) compared to baseline (16.1 ± 9 ng mL^{-1}) (Table 1).

Pain and arm circumference

All participants reported no pain on the VAS at baseline. After exercise, pain values significantly increased at 24 h,

Table 1 Serum creatinekinase, myoglobin, h-FABP, and HMGB1 after eccentric exercise

	Pre-Ex.	Post-Ex.	20 min	2 h	24 h	48 h	72 h
Creatinekinase, U L^{-1}	168.5 ± 137.9	182.5 ± 156.1	182.3 ± 149.7	246.0 ± 193.9	11,927 ± 18,358*	34,993 ± 31,014*	51,898 ± 29,724*
Myoglobin, ng mL^{-1}	1.9 ± 1.4	2.4 ± 1.4	4.2 ± 2.5	11.1 ± 15.4*	78 ± 82.7*	175.5 ± 99.9*	139.4 ± 83.7*
h-FABP, ng mL^{-1}	2.8 ± 0.6	2.8 ± 0.4	3.5 ± 1.2	5.2 ± 4.8	22.9 ± 27*	58.1 ± 33.6*	41.3 ± 23.3*
HMGB-1, ng mL^{-1}	16.1 ± 9	25 ± 10.3*	16.5 ± 8.1	16.2 ± 8	17.8 ± 9.2	16.9 ± 10.2	15.8 ± 7.7

Values are displayed as means ± SD

*Significantly different from Pre-Ex ($p < 0.05$)

48 h, and 72 h ($p < 0.001$) peaking at 48 h (44.4 ± 12.8 mm) (Fig. 3a).

The baseline arm circumference was 30.3 ± 2.5 cm. The arm circumference was significantly elevated at 24 h ($p = 0.012$), 48 h and peaking at 72 h ($p < 0.001$) (Fig. 3b).

Tensiomyography (TMG)

At baseline, the maximal displacement (D_m) of the elbow flexor was 12.4 ± 3.5 mm. D_m decreased significantly immediately after exercise (− 32%, $p = 0.004$), at 20 min (− 38%, $p = 0.004$), at 2 h (− 39%, $p = 0.002$), at 24 h (− 38%, $p = 0.004$) and 72 h (− 35%, $p = 0.006$) (Fig. 3c).

The sustained time (T_s) was significantly longer at 24 h (111.2 ± 20.2, 18.1 ms $p = 0.002$), 48 h (114.1 ± 27.1, $p = 0.003$), and 72 h (110.3 ± 29.3, $p = 0.043$) compared to baseline (77.6 ± 18.1 ms) (Fig. 3d). The relaxation time (T_r) was also significantly longer at 24 h (79.8 ± 21.5 ms, $p = 0.003$), 48 h (82.1 ± 29.6 ms, $p = 0.016$), and 72 h (79.4 ± 23.3 ms, $p = 0.043$) compared to baseline (48.8 ± 18.4 ms). Baseline values of T_d (23.6 ± 2.1 ms) and T_c (24.2 ± 3.2 ms) remained statistically unchanged.

Correlations

Spearman rank correlation coefficients between D_m vs. CK, Mb, h-FABP, HMGB1, arm circumference, and pain are shown in Table 2. Large correlation coefficients ($r \geq 0.7$) were apparent for D_m to CK (Fig. 4b), Mb and h-FABP after 48 h as well as to arm circumference after 20 min ($p < 0.05$). T_c only showed a large correlation to pain after 20 min ($r = 0.71$, $p = 0.027$), T_s to arm circumference after 2 h ($r = -0.62$, $p = 0.049$), and T_r to pain after 2 h ($r = -0.71$, $p = 0.022$). T_d did not correlate to any of the muscle damage markers or functional outcomes at any time.

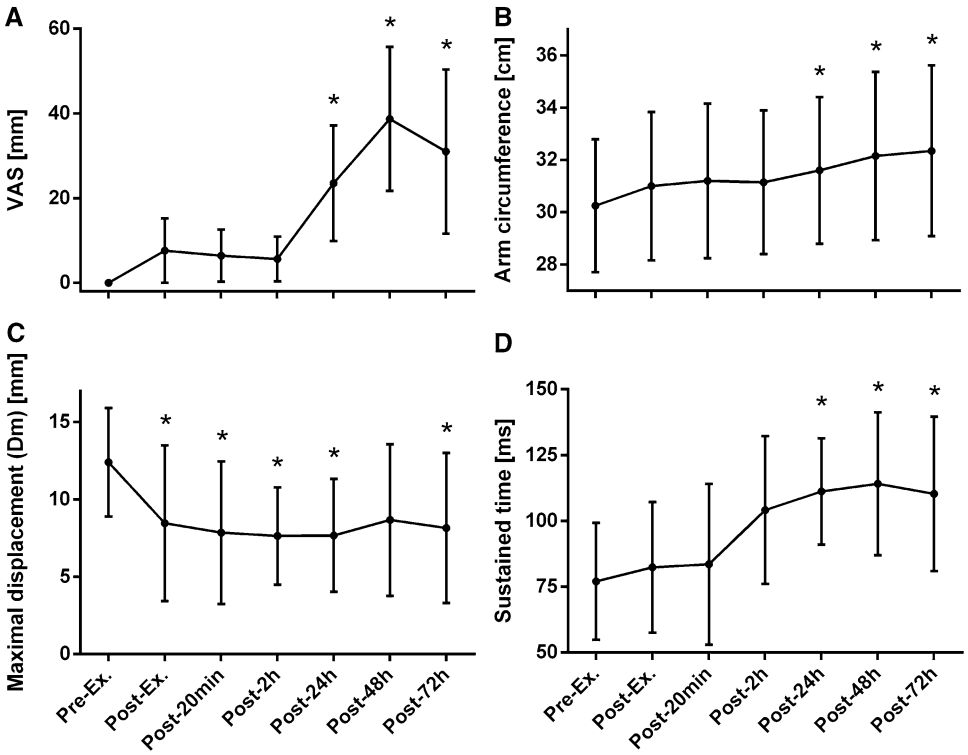

Fig. 3 Overview of perceived pain on the VAS scale (**a**) and arm circumference (**b**) as well as TMG data including maximal displacement (D_m) (**c**) and sustained time (**d**). *Significantly different from Pre-Ex ($p < 0.05$). Vertical bars in figures represent standard deviation

The correlations of h-FABP to other damage markers are shown in Table 3. The correlations for h-FABP to CK and Mb (Fig. 4a) were very large ($r \geq 0.9$) after 24 h ($p < 0.001$) and still large after 48 h ($r \geq 0.7$, $p < 0.01$). In addition, significant correlations between h-FABP to the sensation of pain beginning after 2 h were apparent ($p < 0.05$) with the highest correlation after 48 h ($r = 0.91$, $p < 0.001$) and to HMGB-1 at 48 h ($r = 0.71$, $p = 0.028$). For HMGB1, no further significant correlations to other damage markers existed.

Discussion

The primary goal of the present study was to correlate TMG parameters with four different biomarkers (CK, Mb, h-FABP, and HMGB1) as well as pain perception and muscle swelling after eccentric exercise of the elbow flexors.

Muscle contractility analysis by TMG revealed that, especially, the D_m parameter significantly decreased after eccentric exercise of the elbow flexors. This is in accordance with previous studies and highlights that D_m represents a major indicator of fatigue (Hunter et al. 2012; García-Manso et al. 2012; Franz et al. 2017). As Hunter et al. (2012) found significant correlations of decreased D_m to reductions in MVC torque, this TMG parameter might be similarly indicative for impaired muscle function associated with EIMD. One of the studies using a very similar loading protocol (8 × 8 at 110% 1RM for both arms eccentric only) in resistance-trained young males found isometric torque to be significantly reduced immediately afterwards (26%) and still reduced up to 48 h (Paddon-Jones and Quigley 1997). As we used untrained males, we would expect the reduction in isometric torque to be even greater. Therefore, despite the absence of force measurements in the present study, we assume that muscle function was severely compromised after eccentric exercise as also indirectly indicated by the significant increase in CK, Mb and h-FABP, and arm swelling.

Table 2 Correlation coefficients for % change from Pre-Ex. for maximal displacement (D_m) vs. CK, Mb, h-FABP, HMGB1, arm circumference (Arm c.), and pain (*$p < 0.05$, **$p < 0.01$)

	Post-Ex.	20 min	2 h	24 h	48 h	72 h
CK	−0.74*	−0.66*	−0.42	−0.62	−0.95**	−0.79**
Mb	−0.43	−0.62	−0.19	−0.73*	−0.87**	−0.66*
h-FABP	−0.23	−0.47	−0.31	−0.70*	−0.79**	−0.24
HMGB1	0.25	−0.26	−0.40	−0.35	−0.52	−0.50
Arm c.	−0.55	−0.72*	0.01	−0.25	−0.55	−0.70*
Pain	−0.13	−0.02	−0.30	−0.32	−0.70*	−0.44

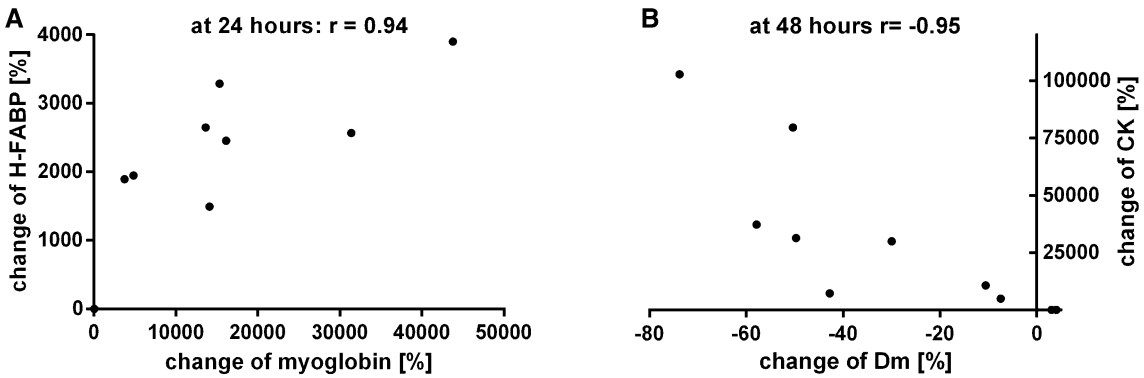

Fig. 4 Individual data of Spearman rank correlation analysis for h-FABP to myoglobin (**a**) and CK to Dm (**b**)

Table 3 Correlation coefficients for % change from Pre-Ex. for h-FABP vs. CK, Mb, HMGB1, arm circumference (Arm c.), and pain. (*$p < 0.05$, **$p < 0.01$)

	Post	20 min	2 h	24 h	48 h	72 h
CK	0.51	0.45	0.79**	0.96**	0.83**	0.42
Mb	0.43	0.02	0.51	0.94**	0.80**	0.55
HMGB1	0.43	0.12	−0.25	0.28	0.71*	0.62
Arm c.	−0.20	0.21	0.60	0.60	0.42	0.21
Pain	0.51	0.23	0.70*	0.80**	0.91**	0.66*

To the best of our knowledge, this study is the first to report a significant increase of the sustained time (T_s) and the relaxation time (T_r) of the biceps brachii muscle after eccentric exercise. Both parameters indicate a prolongation of a single muscle contraction after eccentric exercise, whereas T_r particularly implies a slower relaxation of the muscle. However, the contraction time (T_c) did not change significantly potentially implicating that the Ca^{2+} release to enable the contraction is not affected. Therefore, the slowing in T_s and T_r could rather be explained by an impairment of Ca^{2+}-reuptake by sarco(endo)plasmic reticulum ATPases (SERCA) which has been frequently observed after exhaustive muscle fatigue (Allen et al. 2008).

For the correlation of TMG parameters with frequently used indicators for muscle fatigue and damage, we assessed subjective pain values (VAS 0–100 mm), arm swelling as well as typical (CK, Mb) and new candidate serum biomarkers (h-FABP, HMGB1). Twenty minutes after eccentric exercise, the decline in D_m correlated with arm swelling ($r = -0.72$), indicating that the beginning accumulation of interstitial fluid in the elbow flexors through increased vascular permeability might impair muscle contractility. However, as significant edema formation may take up to 48 h (Chleboun et al. 1998), it is likely that the immediate increase in muscle stiffness rather occurs due to modifications in contractile function. The underlying mechanisms by which the mechanical strain of eccentric loading affects muscle stiffness remain to be investigated. Several studies demonstrated that eccentric contractions led to enhancements in passive muscle stiffness (Xu et al. 2018; Yanagisawa et al. 2015) to potentially promote passive force enhancement or to reduce the strain of later contractions that would otherwise damage the muscle even further. In this context, posttranslational modifications of cytoskeletal proteins and alterations in muscle viscoelasticity are suggested to contribute to post-exercise muscle stiffness. Especially, the potential role of titin can be highlighted (Hessel et al. 2017). Titin stiffness is directly increased by Ca^{2+} influx and force development in active muscle, and through Ca^{2+}-dependent binding of titin to actin its free length becomes reduced by concurrent enhancements in passive muscle stiffness (Herzog et al. 2016). Furthermore, Nishikawa et al. (2012) propose a 'winding filament' hypothesis, whereby cross bridges serve as rotors that wind titin on actin, storing elastic energy in the proline–glutamate–valine–lysine (PEVK) region of titin, which can subsequently contribute to passive muscle stiffness after exercise.

Very large correlations between the D_m parameter and CK (Fig. 4b), Mb, and h-FABP were reported after 48 h. By contrast, whereas Hunter et al. (2012) found similar decreases in D_m after unilateral maximal eccentric contractions of the elbow flexors (D_m of 15.4 ± 0.9 mm at baseline declining to 10.7 ± 0.9 mm after 24 h; mean drop of 31%) and significant correlations of D_m to CK, they showed an additional increase in T_c after exercise associated with less muscle damage (CK values < 800 U L^{-1}). In our study, a larger decrease of D_m (12.4 ± 3.5 mm at baseline to 7.6 ± 3.1 mm after 24 h; mean drop of 39%) and a clearly higher CK response were not accompanied by a significant increase in T_c. A simple reason for this contradictory finding might be that the considerably lower D_m value after 24 h requires much less time to be reached and then a potential decline of T_c might become undetectable. Despite the significant rises in T_s and T_r, no substantial correlations were observed to serum or even functional outcomes. These results suggest that muscle damage processes did not affect those two temporal parameters.

In the evaluation of alternative muscle damage markers, especially, the almost identical increase after 24 h of h-FABP, CK, and Mb was remarkable. Although h-FABP is known to be mainly expressed in cardiac muscle, its accessory release from skeletal muscle cells is well reported after marathon running (Scherr et al. 2011), submaximal sprinting (Behringer et al. 2017a) as well as after eccentric accentuated knee extensions (Behringer et al. 2017b). Since h-FABP and Mb nearly share the same molecular size (~ 15 and ~ 17 kDa) allowing the direct transepithelial transition into the bloodstream, they are known to show similar serum response curves after muscle injury (van Nieuwenhoven et al. 1995) (Fig. 4a). Mostly due to its larger size, CK (~ 84 kDa) is known to migrate into the interstitial fluid, where it may enter the blood through the lymphatic system leading to later serum peak values than Mb (Driessen-Kletter et al. 1990) and h-FABP. Correlations of h-FABP to CK and Mb were still significant after 48 h but not after 72 h. This indicates a potentially faster renal and/or hepatic elimination pathway of h-FABP compared to CK and Mb. Besides the strong correlations to D_m (see Table 2), h-FABP was also correlated with pain perception beginning after 2 h. Since h-FABP has already been shown to be useful as a point-of-care-testing (POCT) system in the early detection of cardiac damage (Li et al. 2010), our findings could be relevant for practitioners in athletic training to obtain rapid information about the degree of muscle damage. Although CK can also be assessed by POCT systems (Peñailillo et al. 2013), h-FABP possesses a higher practical value due to its earlier rise in plasma in combination with the large correlations to early changes in TMG, thereby providing faster feedback about muscle affection and damage within hours.

Regarding the new candidate biomarker HMGB1, which is known to be associated with polytrauma and subsequent muscle repair (Peltz et al. 2009; Venereau et al. 2013), we found a significant rise only immediately after exercise. This result is in accordance with outcomes reported by Beiter et al. (2011) who found a similar significant increase of HMGB1 immediately after exhaustive short-term treadmill exercise dropping back to resting values after 30 min. By contrast, Behringer

et al. (2016) did not find a detectable increase of HMGB1 after 100 drop to vertical jumps as well as after a 1200-km bicycle race. Based on the present and previous findings, it can be argued that a sustained rise of HMGB1 might only be detectable after more complex mechanical traumata involving several types of tissues rather than skeletal muscle damage alone (Peltz et al. 2009). As we found no correlations between HMGB1 and TMG parameters, we conclude that HMGB1 might rather be unsuitable for exercise science purposes to quantify EIMD over time.

Other studies investigating eccentric EIMD and serum markers showed that even in very homogenous samples, there are some individuals showing a high response in terms of increasing CK after exercise, and others demonstrating a low or no response at all, called nonresponders (NR) (Kim and Lee 2015; Clarkson et al. 1992). In this regard, it is interesting to report that two individuals of our sample could be classified as NR. Whereas the other eight high responders reached CK values of at least 40.000 U L^{-1}, the two NR remained within the resting range below 150 U L^{-1}. These two NR also did not demonstrate any increase of Mb and h-FABP above the resting range over the examined post-exercise period. However, HMGB1 was elevated in both NR immediately after exercise contributing to the significant increase of the whole sample. Therefore, even in NR, cell homeostasis seems to be impaired after eccentric exercise. This is supported by a concurrent increase of perceived pain and altered contractility patterns (increase of T_r and T_s) in both NR. By contrast, while D_m of all high responders stayed significantly below baseline values at almost all post-measurements representing an impaired or rather altered contractility of the elbow flexors, D_m of both NR even slightly increased immediately after exercise and declined only after 20 min or 2 h but fully recovered already after 48 h. This finding of enhanced contractility after exercise seen in the two NR is quite similar to findings from Hubal et al. (2007), who identified three out of 46 participants as NR demonstrating a greater post-exercise contractility assessed by MVC than before eccentric contractions of the elbow flexors. However, due to the very small number of NR in our study and others, these findings have to be treated with caution. Future studies should try to seek out potential NR accumulating them to a greater sample size and subsequently carry out detailed investigations, like, e.g., genetic screenings, to determine further common characteristics of NR as the less impaired muscle contractility. For this purpose, TMG might also be a cost-effective tool to identify potential NR.

Conclusion

In conclusion, the present study indicates that TMG data correlate with muscle damage markers after eccentric exercise of the elbow flexors of untrained males. Therefore, we support the use of TMG as a non-invasive and cost-effective alternative to muscle damage markers to quantify the muscle damage response of individual patients and athletes to specific exercise interventions. Independent of the extent of muscle damage, a decrease of D_m seems to be a reliable finding after eccentric exercise. Additional increases of T_r or/and T_s could indicate pronounced impairment of muscle contractility and further rest may be needed for the individual to recover. For field application, the less frequently used biomarker h-FABP might be a promising alternative for the early monitoring of EIMD. Although demonstrating similar kinetics as CK and Mb, h-FABP might be superior due to its earlier rise in plasma than CK and its possible application in POCT contrary to Mb.

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Compliance with ethical standards

Conflict of interest All authors declare that there are no financial and personal relationships with third parties or organizations that could have inappropriately influenced the present work. The authors further state that no funding was received.

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